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Identification of Mosaicism in Prader-Willi Syndrome Using Fluorescent In Situ Hybridization

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We report on our findings of 4 patients with mosaicism for a deletion of chromosome 15, most commonly associated with Prader-Willi syndrome (PWS). We examined a series of typical and atypical PWS patients in order to identify cytogenetically undetected deletions, using fluorescence in situ hybridization. In 4 of the patients analyzed we detected a deletion in 14–60% of peripheral blood leukocytes, using four commercially available probes. Our results indicate that mosaicism may play a role in the etiology of some PWS cases. These findings may be especially useful in patients who display discrepancies between clinical phenotype and established diagnostic criteria. Methylation and microsatellite polymorphism analyses of 2 patients with low-level mosaicism failed to identify the deletion. We propose that fluorescence in situ hybridization is the most effective method for detecting somatic mosaicism, since a large number of cells can be individually examined for the presence or absence of a specific deletion.

KEY WORDS: Prader-Willi syndrome, somatic mosaicism, fluorescence in situ hybridization

INTRODUCTION

Prader-Willi syndrome (PWS) is a complex genetic disorder affecting approximately 1/20,000 newborns [Cassidy, 1984]. It is characterized by hypotonia and feeding difficulties in early infancy, followed by increased appetite and rapid weight gain resulting in obesity, usually by early adolescence. Other characteristics include developmental delay, hypogonadism, short stature, small hands and feet with tapering digits, characteristic facial findings, and very characteristic behavioral problems [Holm et al., 1993]. The association between PWS and a specific chromosomal abnormality was not established until 1981 [Ledbetter et al., 1981]. Since then, it has been shown that approximately 70% of PWS patients have a deletion of the proximal q arm of chromosome 15 at bands q11–q13 [Cassidy et al., 1984]. The size of the deletion varies among affected individuals, and in many cases cannot be detected cytogenetically [Delach et al., 1994; Butler, 1995]. The remaining 30% do not have a detectable deletion, but meet all of the other major diagnostic criteria that characterize the syndrome [Mascari et al., 1992]. Most of these cases have been shown to be the result of maternal uniparental disomy (UPD). A small number of familial cases appear to be the result of microdeletions in an imprinting center (IC) 5′ to the *SNRPN* gene [Buiting et al., 1995].

The diagnosis of PWS has challenged clinicians since it was first described in Prader et al. [1956]. The clinical presentation in infancy is characteristic of many different genetic disorders. The diagnostic findings of PWS change dramatically in early childhood and can be complicated by the wide variation in phenotypic expression found among affected individuals. Laboratory investigation is complicated by several factors. First, the deletion is often not detectable using standard or high-resolution cytogenetic analyses. Second, the deletion, once identified, may not appear to be present in all cells because both homologues may not be sufficiently elongated for separation of bands 15q11 and 15q12. Third, there have been reports in the literature describing heterogeneity of the 15q11–q13 region leading to ambiguous diagnoses or misdiagnosis of a deletion [Wyandt et al., 1981; Kaplan et al., 1987; Trent et al., 1991; Saitoh et al., 1994; Delach et al., 1994; Woodage et al., 1994; Butler, 1995]. Subsequent DNA polymorphism analysis using multiple DNA probes and/or fluorescence in situ hybridization (FISH) studies showed that this region was not deleted in many of the cases described.

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Molecular genetic analysis has proven very useful in the further delineation of the etiology of PWS. Extensive molecular genetic studies of affected individuals and their families have narrowed the critical region to approximately 130 Kb, between *D15S63–SNRPN* [Buiting et al., 1995]. Analysis of the methylation patterns of imprinted genes has proven useful in the accurate diagnosis of most PWS, especially in prenatal and neonatal cases [Gillessen-Kaesbach et al., 1995]. However, methylation analysis provides diagnosis which does not differentiate patients with deletions from those with uniparental disomy.

Cytogenetic studies combined with FISH analysis have proven useful in the rapid detection and confirmation of deletions in suspected PWS/AS patients [Delach et al., 1994]. In cases without a deletion, further testing can then be done in order to confirm or exclude uniparental disomy. In some instances, neither a deletion nor UPD is found and the diagnosis may be called into question, or further molecular analyses may be warranted. This report documents mosaicism as another etiology that leads to PWS and may not be easily detected by DNA polymorphisms, methylation, or high-resolution cytogenetic studies. We report on typical and atypical patients with PWS in whom mosaicism for the deletion was identified by FISH.

MATERIALS AND METHODS

Studies were carried out on a series of 16 patients who were referred to the Rehabilitation Institute in Pittsburgh for obesity and behavioral problems related to Prader-Willi syndrome. This group of patients was referred from May 1994–September 1995. Each patient was selected for this study based on one of two criteria: 1) atypical features for PWS; or 2) typical features for PWS, but without a cytogenetically detected deletion. A summary of clinical and molecular cytogenetic findings is included in Table I. A detailed clinical description of each of the 4 mosaic patients is presented below.

CLINICAL REPORTS

Patient PW1

This 39-year-old white female was referred for marked obesity and severe behavioral problems (Fig. 1). Little is known about this woman's early childhood, although the pregnancy, labor, and delivery were reported to be normal. She was developmentally slow, with delayed milestones and an IQ in the 60's. She had onset of menses at age 12 years and has been reported to be sexually active. She has been observed to vomit on at least one occasion and has a history of skin picking.

This patient was first institutionalized at age 24 years because of aggressive behavior and multiple episodes of running away, requiring police involvement. She has also had problems with hallucinations and psychotic episodes. She hoards food and continues to gain weight due to excessive overeating. The family history was significant for short stature and mental retardation. Her mother is reported to have "mental health problems." She also has 4 sibs. Two older brothers are institutionalized. One brother lives in a group home for the mentally retarded. The other brother is apparently severely mentally retarded, blind, and deaf. Two sisters are reportedly normal.

Physical examination showed a moderately retarded-appearing woman who had a facial appearance compatible with Prader-Willi syndrome, but not typical. Her mouth was somewhat small, but her eyes were not almond-shaped and her bifrontal diameter was normal. Other atypical features included normal genitalia and hirsutism. She had small hands and feet and was massively obese. Her weight was 118.2 kg (>95th centile), and her height was 157.1 cm (10th centile). Previous cytogenetic studies did not detect a deletion on chromosome 15. An ultrasound evaluation for possible Stein-Leventhal syndrome showed a rather thin endometrium and very small ovaries, but no evidence of polycystic ovaries. MRI studies indicated that she has partial Empty Sella syndrome; however, this is not considered clinically significant.

TABLE I. Summary of Clinical Manifestations*

Case	Sex	Age	Obesity (%)	Short stature	MR	Hypo-gonad	Typical facies	Behav-ioral problem	Small H/F	Hypo-tonia	Skin picking	Hx vomit	Cyto	FISH
PW1	F	39	140	−	+	−	−	+	+	N/A	±	+	−	±
PW2	M	22	200	+	+	−	+	+	+	+	+	−	+	±
PW3	M	23	68	−	++	+	+	+	−	+	−	−	+	+
PW4	M	13	22	±	−	−	+	±	+	+	+	+	−	±
PW5	F	12	42	+	+	−	+	+	+	+	+	N/A	−	+
PW6	M	42	113	−	+	+	+	±	−	N/A	−	N/A	N/D	−
PW7	M	11	100	−	+	−	−	+	+	+	+	+	N/D	−
PW8	M	10	100	−	+	−	−	+	+	+	+	N/A	−	−
PW9	F	30	170	+	+	−	+	+	±	+	−	−	N/D	+
PW10	F	18	40	−	±	−	−	+	−	+	++	+	−	±
PW11	F	30	300	+	+	−	+	+	+	+	+	−	+	+
PW12	F	37	300	+	+	−	+	+	+	+	+	−	−	+
PW13	M	12	33	±	+	−	−	++	+	+	+	N/A	+	+
PW14	M	16	150	−	+	+	−	++	−	+	±	+	−	−
PW15	M	21	200	+	±	+	+	+	+	+	±	N/A	−	−
PW16	M	42	215	−	±	+	−	+	−	N/A	−	−	−	−

* MR = mental retardation; small H/F = small hands and feet; cyto = deletion detected by standard cytogenetics; FISH = deletion detected by FISH; + = feature observed; − = feature not observed; ± = mosaicism observed; N/A = information not available; N/D = testing not done.

Fig. 1. Patient PW1. Atypical findings include: normal height, eyes not almond-shaped, normal bifrontal diameter, hirsutism, normal genitalia, development of secondary sex characteristics. She does display some characteristic findings of PWS, such as: massive truncal obesity, small hands and feet, mental retardation, skin picking, and behavioral problems.

Fig. 2. Patient PW2. Classical findings include: narrow bifrontal diameter, almond-shaped eyes, downturned corners of the mouth, small hands, truncal obesity, and fair hair and complexion. Abnormal position of his head is due to a cervical laminectomy for stenosis of the cervical canal.

Patient PW2

This is a 22-year-old white male (Fig. 2). He has a typical history of Prader-Willi Syndrome with early hypotonia and poor feeding, followed by development of a voracious appetite with marked weight gain. At age 14 years he weighed 247 pounds and had frequent temper tantrums and aggressive behavior. In the past 2 years he has had psychotic episodes with hallucinations. As obesity increased, he developed shortness of breath and episodes of sleep apnea. Family history was not significant for any similar syndromes. He has one older sister who is reportedly normal.

Physical examination showed a morbidly obese young man who appeared younger than his stated age. His weight was 152.4 kg (>95th centile), and his height was 159.9 cm (50th centile for 13.5 years). He had exceptionally blond hair and very blue eyes, a narrow bifrontal diameter, almond-shaped eyes, downturned corner of the mouth, small hands and feet, and small genitalia. Previous cytogenetic studies reportedly showed a deletion of chromosome 15q11q13.

Patient PW4

This 13-year-old white male was referred for marked weight gain. He was the product of an 8-month pregnancy and weighed 5 pounds at birth. He had been hypotonic, with a poor suck reflex and a weak cry, but was able to breast-feed for the first 7 months. Developmental milestones were not delayed. His appetite was below normal until age 4, at which time his weight was <5th centile. Congenital heart disease (VSD) was found in the preschool years. He has not required surgery. By age 9 years his weight was at the 75th centile. He is reported as not having an overly voracious appetite, but is very interested in food.

He attends a regular school and is able to maintain a C average. He displays some behavioral characteristics of PWS. He is argumentative and stubborn, and has a very rigid personality. He also has a significant problem with skin picking. He does not demonstrate aggressive behavior or temper tantrums. He is reported to have vomited on several occasions, but fewer times than his brother. Family history was negative.

Physical examination showed a short boy measuring 133 cm (<5th centile for age 9 years) with moderate obesity (43.2 kg, >95th centile). He had blond hair and blue eyes and typical facial and bodily characteristics of PWS, including almond-shaped eyes, narrow bifrontal diameter with a thin upper lip and downturned corners of the mouth, and small hands and feet. He did not have hypogonadism. Cytogenetic studies were performed at age 3 weeks to rule out Down syndrome. Results were reportedly normal. A cytogenetic study was repeated in 1993 and did not detect a deletion. Uniparental disomy testing was also performed. Results of DNA polymorphism analyses indicated that the patient inherited one chromosome 15 from each parent. Analysis of the methylation pattern of the *SNRPN* upstream reading frame confirmed biparental inheritance.

Patient PW10

This 18-year-old white female was referred for progressive behavioral problems and the recent development of mild obesity. She was diagnosed with Prader-Willi syndrome following an endocrine evaluation in 1994. She was the product of a full-term pregnancy, delivered by forceps. Birth weight was 6 pounds, 7 ounces. She was extremely hypotonic at birth, but was noted to have had a good suck reflex and appetite, and she gained weight well. She had a normal cry and appeared to cry more frequently than other infants. Developmental milestones were delayed, with sitting at age 8 months and walking at age 2 years.

She attends special education classes for the mildly mentally retarded and is doing well. She is able to ride a bicycle. She is very stubborn and argumentative, and began to have temper tantrums in her preschool years. She has chronic skin-picking problems. She has had several episodes of vomiting throughout her lifetime. She also displays marked food-seeking behavior. She has one younger half-brother who is reportedly normal. Family history was negative.

Physical examination showed a well-developed, attractive young woman who has facial findings consistent with Prader-Willi Syndrome but not classical. Her height is 154 cm (5th centile), and she is taller than her mother. Her weight is 65 kg (80th centile). She does not have a narrow bifrontal diameter, and her face is fuller and her nose and mouth more prominent than most PWS patients, but her eyes are somewhat almond-shaped. She has two café-au-lait spots. Other atypical findings include normal hands and feet, normal female genitalia, breast development at age 12, onset of menses at age 14, and hirsutism. Subsequent mammoplasty was required to reduce breast size. She has been on birth-control pills because of irregular menses and thinning of scalp hair. An extensive workup including multiple endocrine tests, chemistry screen, and CBC were normal. Cytogenetics showed a normal female karyotype. Analysis of the methylation pattern of the *SNRPN* promoter did not detect a deletion, and indicates biparental inheritance of alleles on chromosome 15.

METHODS

Molecular cytogenetic studies were performed in order to confirm or exclude the diagnosis of Prader-Willi syndrome. High-resolution chromosome banding was carried out in order to confirm previous cytogenetic results, or to determine whether a deletion was evident in patients who had not been previously tested. Chromosome preparations were made using standard techniques [Gosden et al., 1992; Seabright, 1971].

FISH was performed using digoxigenin-labeled DNA probes (Oncor, Inc., Gaithersburg, MD) following the manufacturer's recommended protocol. Each patient was tested with up to four different probes which map to chromosome 15q11–q13. The probes were *D15S11* (region A), *SNRPN, D15S10*, and *GABRβ3* (region B). Both an internal and external control were examined with each individual hybridization. The internal control was provided by Oncor, Inc., which includes a control chromosome 15-specific probe, *PML*, incorporated into the hybridization mix. This allows not only for the identification of both of the chromosome 15 homologues, but also for assessment of the quality of hybridizations among different samples. An external control, prepared from a normal (non-PWS) individual, was also hybridized with each sample. The use of the external control allowed us to continuously monitor the hybridization efficiency of each probe and detect substandard hybridization reactions due to either probe quality or technical problems. In addition, we established strict scoring criteria for reporting normal and abnormal (deleted) results. We considered a 5% error (inconsistency) rate (1/20) to be acceptable. If two or more of 20 cells were not consistent, an additional 30 cells were examined (when available). During the course of this study we examined numerous normal control and patient samples, and showed a consistent hybridization efficiency ≥99% with all four probes (Table II).

A minimum of 20 cells was examined for each probe and scored for the presence or absence of a deletion.

TABLE II. Summary of FISH Results for Patients With Mosaic Deletions*

Locus	PW1	PW2	PW4	PW10	Nondel PWS[5] (pooled)	Del PWS[5] (pooled)	Controls[5]
D15S11	16/50[1]	31/53	0/20	0/20	0/40	40/40	0/184
	32%	58%			N = 2[3]	N = 2	N = 11
SNRPN	21/71	44/72	0/20	0/20	0/121	158/160[4]	4/568
	30%	61%			N = 6	N = 6	N = 29
						1.3%	<1%
D15S10	2/46[2]	41/65	13/89	9/65	2/60	112/114	0/78
	4%	63%	15%	14%	N = 3	N = 5	N = 5
					3%	1.7%	
GABRβ3	23/70	46/75	0/20	0/20	0/41	80/80	3/325
	33%	61%			N = 2	N = 4	N = 18
							<1%

*Numbers in superscript indicate: 1 = Number of deleted cells/total cells examined; 2 = False positive result not significant if ≤5%; 3 = Number of individuals included in pooled samples; 4 = False negative result not significant if ≤5%; 5 = To establish the efficiency of the hybridization and detection, the following control specimens were tested: patients with a diagnosis of PWS without prior evidence of a chromosome deletion (Nondel PWS), patients with a previously established deletion (Del PWS), and normal control individuals (Controls).

Each cell examined contained two identifiable chromosome 15s, as demonstrated by the presence of marker locus *PML* at 15q22. Each patient and control sample was evaluated and checked by 2 different individuals. Discrepancies were carefully reviewed, and hybridization was repeated if necessary. A deletion was reported when the 15q11q13-specific probe was consistently absent (≤5% error) from one of the chromosome 15 homologues. Polymorphic satellite variants were recorded to insure accurate scoring of all cells examined. This was especially important in order to confirm that the deletion was always occurring on the same homologue, and that loss of signal was not due to poor hybridization conditions or differential condensation of the chromosomes. Mosaicism was diagnosed when some cells were found in which both homologues displayed the 15q11q13-specific signal and the *PML* marker signal, while in other cells one of the chromosome 15 homologues did not display the 15q11q13-specific signal.

In the four cases in which mosaicism was detected, we examined at least 50 cells for each of the loci involved. This is based on the calculation that a minimum of 50 cells should be analyzed to detect ≥6% mosaicism with a 95% confidence interval [Hook, 1977]. In addition, in the first two cases, two sets of blinded slides were independently evaluated by 2 different technologists, and an additional set of blinded slides was sent to another laboratory for independent confirmation of the FISH results (by Sharon L. Wenger, Children's Hospital of Pittsburgh). In each case, the mosaic individuals were correctly identified and the level of mosaicism was consistent.

Two additional types of analyses were performed on the other two mosaic cases: 1) DNA polymorphism analysis to determine the inheritance of parental alleles (PW4); and 2) studies to look at methylation patterns of the promoter region of *SNRPN* (patients PW4 and PW10). Genomic DNA was extracted from purified leukocytes from each patient and his/her parents using a nonorganic procedure [Miller et al., 1988]. The inheritance of parental alleles was evaluated at 12 polymorphic chromosome 15 loci, including seven markers that occur within the common interval found in the majority of patients with paternally-derived deletions. These included: *D15S18, D15S11, D15S128, D15S113, D15S97, GABRβ3, GABRβ5, D15S119, D15S125, D15S111*, and *D15S107*. These genetic markers were amplified by polymerase chain reaction and analyzed according to previously established procedures [Weber and May, 1989]. A genetic map of this region is provided (Fig. 3).

For methylation analyses, genomic DNA was digested with restriction enzymes *Xba*I (methylation-sensitive) and *Not*I, and hybridized with a DNA probe from the promoter region of the *SNRPN* gene. DNA methylation in this domain is distinctive in patients with PWS, since methylation of the inactive maternally-derived signal is detected in the absence of the paternal signal. Normal individuals display genomic fragments of 4.2 Kb and 0.9 Kb, whereas individuals with PWS exhibit only the methylated 4.2-Kb maternally-derived band [Buiting et al., 1995].

RESULTS

Results of the FISH analyses are summarized in Table II. The 4 patients (PW1, PW2, PW4, and PW10) were found to be mosaic for a deletion of chromosome 15 at q11q13. Patient PW1 was deleted at 3 of the 4 loci examined in approximately 30% of her cells (Fig. 4). The fourth locus, *D15S10*, which is located internally to the other loci, did not appear to be deleted. These results indicate two possibilities: 1) a submicroscopic rearrangement occurred prior to the deletion; or 2) two distinct deletions have occurred in this region. However, the exact nature of this rearrangement has not been determined. Patient PW2 demonstrated a deletion of all four of the loci in 60% of his cells. This was surprising because he displayed a classical phenotype and had a cytogenetically-detected deletion which was not noted to be mosaic.

The third and fourth mosaic cases, PW4 and PW10, appeared to be deleted at only one of the loci examined. The *D15S10* locus was deleted in approximately 15% (13/89) and 14% (9/65) of their cells, respectively (99% confidence interval) [Hook, 1977]. Previous high-resolution cytogenetic studies did not detect a deletion in either of these patients. Analysis of microsatellite repeat polymorphisms for multiple genetic markers in the 15q11q13 region using polymerase chain reaction were undertaken in patient PW4 and his parents. Results of these studies are shown in Table III and indicate that heterozygous and biparental inheritance was found at several of the loci, thus excluding uniparental disomy as a possible mechanism for PW4's PWS-like phenotype. Examination of the genotypes for loci in close proximity to the *D15S10* locus, *D15S113* and *D15S97*, were noninformative and do not offer an opportunity to evaluate further the possibility of a small deletion in this region, most likely in a mosaic form. Results of the analysis of the methylation of the *SNRPN* promoter showed that both PW4 and PW10 have a methylation pattern consistent with normal individuals (4.2 and 0.9 Kb bands are present; Fig. 5).

DISCUSSION

The effects of mosaicism are dependent on several factors: 1) stage of development in which the second cell line arises; 2) cell type and stage of differentiation; and 3) type of gene affected (housekeeping, regulatory, tissue-specific, etc). All of these factors will play a role in the severity of the genetic disorder. There are many examples of somatic mosaicism cited in the literature which give rise to variable or incomplete phenotypic expression of a known genetic defect, such as ornithine transcarbamylase deficiency [Maddalena et al., 1988], Smith-Magenis syndrome [Patel et al., 1992; Gardner et al., 1994], epidermolysis hyperkeratosis [Moss et al., 1995; Paller et al., 1994], neurofibromatosis type I [Lázaro et al., 1994; Colman et al., 1996], and Beckwith-Weidemann syndrome [Bischoff et al., 1995]. Mosaicism in Prader-Willi syndrome is rare, and only a few cases have been described in the literature. Most of these involved multiple abnormal cell lines [Lejuene

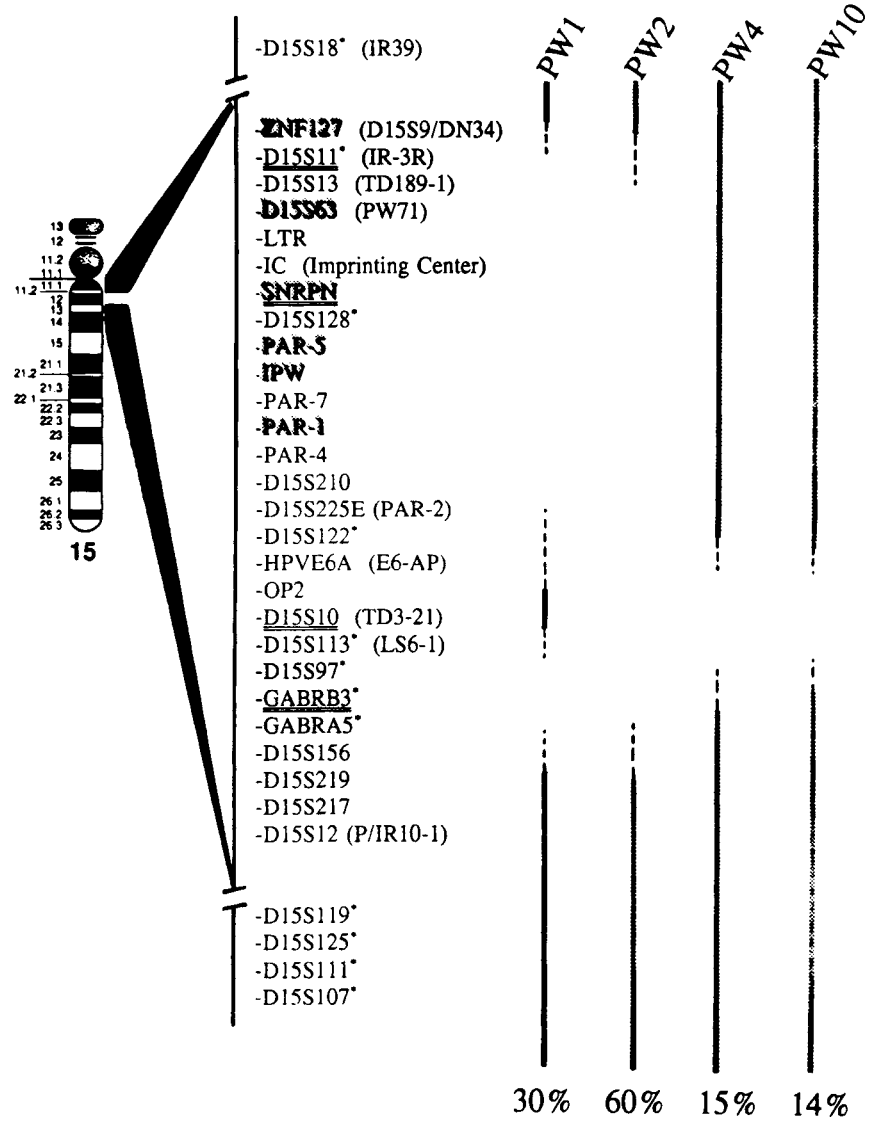

Fig. 3. Genetic map of chromosome 15q11q13. Expanded region represents smallest region of deletion overlap and surrounding loci on chromosome 15. Known extent of the deletion in each patient is indicated by each discontinuous line. Exact breakpoints have not been determined. Percentage at bottom of each line represents percentage of cells found to be deleted in peripheral blood leukocytes. Stippled loci, known imprinted regions; double-underlined loci, loci examined with FISH probes. *Polymorphic markers examined for parental origin.

et al., 1979; Wulfberg et al., 1982; Kousseff et al., 1987; Park et al., 1991] or the presence of a marker chromosome which was usually found to be derived from chromosome 15 [Michaelson et al., 1979; Kousseff, 1980, 1982; Tajara et al., 1982; Ledbetter et al., 1982; Goh et al., 1984; Wenger et al., 1987].

Cassidy et al. [1984] described 2 patients who appeared to be mosaic for chromosome 15 deletion using high-resolution prometaphase-banding techniques. This report was the first to describe mosaicism in PWS, and the authors suggested that mildly affected individuals may exist who have a low level of mosaicism for del(15)(q11–q13) but who do not carry a diagnosis of PWS. The deletion was seen in 36% (n = 11) and 42% (n = 31) of the cells examined; however, the clinical manifestations of these 2 patients did not differ from the rest of the patient population studied. In fact, all of the PWS patients included in the previously mentioned studies were diagnosed as classical PWS and displayed all of the clinical manifestations previously described [Holm et al., 1993]. The strict adherence to these diagnostic criteria eliminated the possible inclusion of misdiagnosed patients in these studies. However, it did not

Fig. 4. Fluorescence in situ hybridization analysis of patient PW1 with *SNRPN* probe. **A**: Representative metaphase spread in which both chromosome 15s are intact at the *SNRPN* locus. **B**: Second metaphase spread, in which one chromosome 15 (prominent satellites) is deleted at the *SNRPN* locus. *PML* is a DNA probe used as a marker in order to identify the presence of both chromosome 15s.

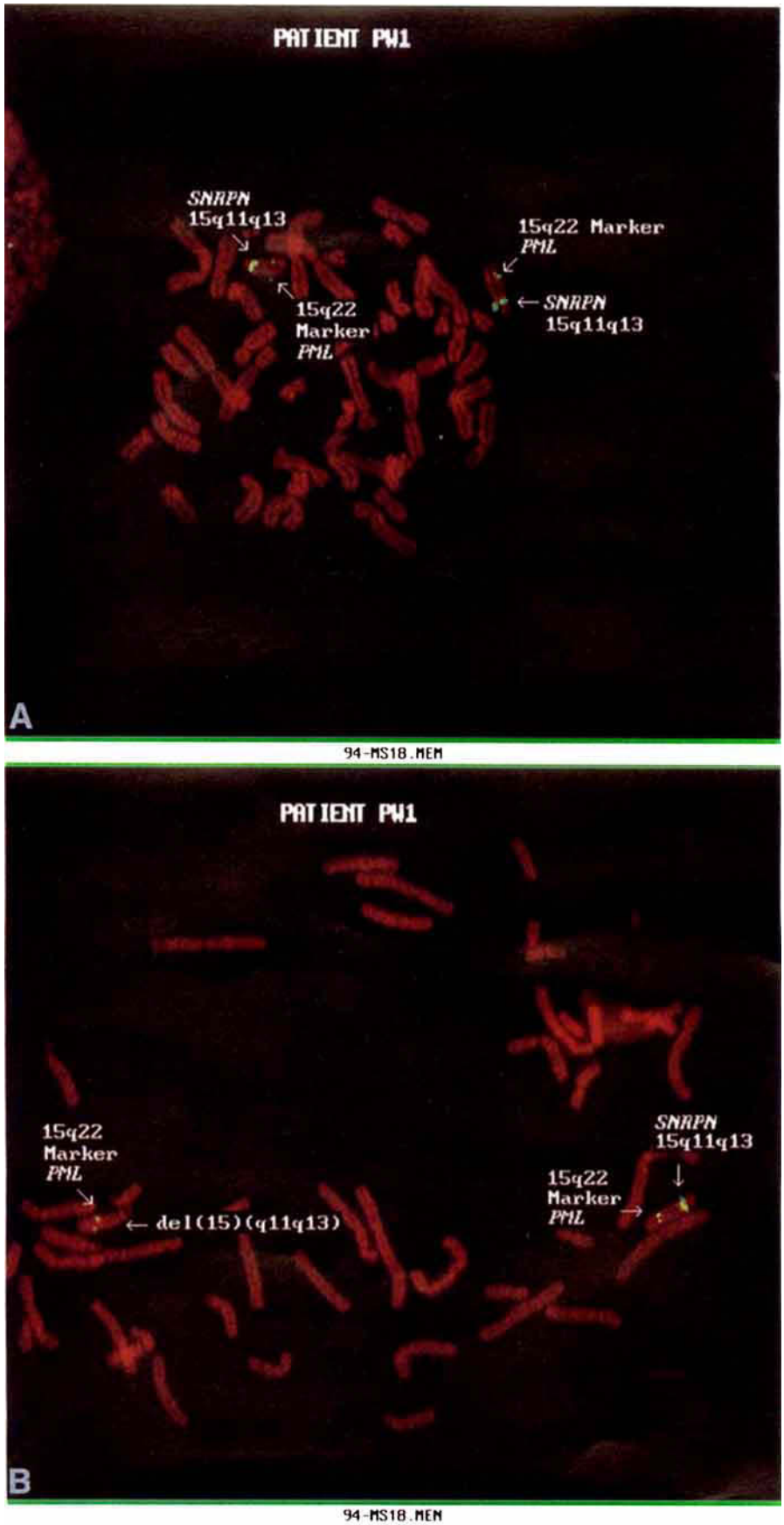
PATIENT PW1
SNRPN
15q11q13
15q22 Marker
PML
15q22
Marker
PML
SNRPN
15q11q13
A
94-MS18.MEM
PATIENT PW1
15q22
Marker
PML
del(15)(q11q13)
SNRPN
15q11q13
15q22
Marker
PML
B
94-MS18.MEM

Fig. 4.

TABLE III. Inheritance of Polymorphic Marker in PW4

	Allele genotypes			
Genetic locus	Father	PW4	Mother	Interpretation
D15S18	13	12	23	Heterozygous, maternal allele informative
D15S11*	12	11 or 1−	12	Uninformative
D15S128*	11	11 or 1−	11	Uninformative
D15S122*	13	23	23	Heterozygous, maternal allele informative
D15S113*	11	11 or 1−	12	Uninformative
D15S97*	11	11 or 1−	11	Uninformative
GABRβ3*	13	13	12	Heterozygous, paternal allele informative
GABRα5*	12	24	34	Biparental inheritance
D15S119	23	12	12	Heterozygous, maternal allele informative
D15S125	12	11	11	Uninformative
D15S111	23	33	13	Uninformative
D15S107	12	13	33	Biparental inheritance

*Indicates genetic markers commonly deleted in patients with PWS.

allow for a milder phenotype which may be an indicator of mosaicism.

At the other extreme, mosaicism may not produce any clinical features. Gonadal and somatic mosaicism has been demonstrated in "cryptic" carriers of hemophilia A [Bröcker-Vriends et al., 1990], Duchenne muscular dystrophy [Bunyan et al., 1994, 1995], Ehlers-Danlos syndrome [Kontusaari et al., 1992], and osteogenesis imperfecta [Wallis et al., 1990]. All of these individuals were asymptomatic for their disorders but transmitted the mutant gene to their offspring. In 1992, two independent reports suggested the occurrence of gonadal mosaicism in several cases of familial del(15)(q11–13) [Örstavik et al., 1992; Patil et al., 1992]. The phenotypes of the carriers ranged from normal to mild developmental delay. However, the affected children demonstrated most of the features of Prader-Willi syndrome.

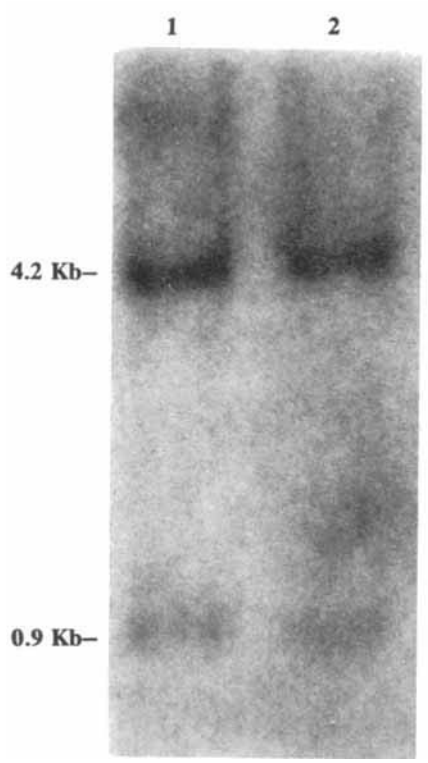

Fig. 5. Methylation analysis of 5′ *SNRPN* promoter. The 4.2- and 0.9-Kb bands represent the maternally and paternally derived alleles, respectively. **Lane 1**, patient PW10. **Lane 2**, patient's mother. The patient and her mother demonstrate both the 4.2- and 0.9-Kb bands, which represent biparental inheritance of the alleles. The patient's father also demonstrated both bands (data not shown).

In our study, the phenotypes of 4 mosaic patients range from classical PWS → atypical but probable PWS → atypical non-PWS. They have variations in both the size of their deletions and the proportion of deleted cells detected in peripheral blood leukocytes. Patient PW1 does not display the full spectrum of this syndrome and was diagnosed with atypical PWS, probable non-PWS. The atypical presentation may be due to the presence of a normal cell line. Patient PW2 displays all of the classical features of PWS, but only has a deletion in 60% of his peripheral blood leukocytes. In this case the proportion of deleted cells may be much higher in other tissues. The remaining 2 patients, PW4 and PW10, are not deleted at the *SNRPN* locus, but appear to have the same smaller deletion in approximately the same percentage of their cells. This is perhaps one of the most important findings in this study. It indicates either that there may be another imprinting center near *D15S10*, or that there are additional critical genes located within the interval between *SNRPN–D15S10* that are required for expression of PWS. Analysis of microsatellite repeats in PW4 does not exclude the possibility that the deletion could include multiple loci between *D15S122–GABRβ3*. No clinical correlation can be made between genotype and phenotype without knowing the percentage of deleted cells in additional tissue types, and a more accurate determination of the size of the deletion. However, our results on PW2 demonstrate that full phenotypic expression may not require that the deletion be present in 100% of cells. Additional

studies involving these patients are being considered. These may include use of different FISH probes to examine the extent of each deletion; testing of additional tissue types if they become available; and further family studies using other markers mapped to this region. These studies would depend on the availability of additional blood and skin samples from each patient and parental cooperation.

In our study, fluorescence in situ hybridization analysis proved useful in identifying 4 PWS-like patients who were mosaic for a deletion involving chromosome 15q11–q13. The use of traditional cytogenetics and/or molecular genetics did not detect the mosaic deletion in these patients. Ascertainment of a mosaic deletion by either minisatellite repeats or methylation would be difficult due to the presence of the normal cell line, which would mask the appearance of the deleted chromosome 15. These are the first reported cases of mosaic PWS which have been detected using FISH. The use of FISH in the diagnosis of microdeletion syndromes offers a rapid diagnosis in cases of full and mosaic deletions. If the patient is chromosomally normal and a deletion is not detected using FISH, then additional testing would be required in order to confirm the diagnosis. Mosaicism, detected by FISH, has not been reported in other contiguous gene syndromes, except Smith-Magenis syndrome [Patel et al., 1992; Gardner et al., 1994] and DiGeorge anomaly (CATCH 22) [Consevage et al., 1996]. We would expect that as the number of individuals screened increases, these cases will be identified.

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